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GBV-C/HGV Infection in Hepatitis C Virus-Infected Deferred Swedish Blood Donors

Steven Shev,[1,2]* Per Björkman,[3] Gunnar Norkrans,[1] Ulla Foberg,[4] Aril Frydén,[4] Gudrun Lindh,[5] Annika Lindholm,[6] Ola Weiland,[5] and Anders Widell[3]

[1]*Department of Infectious Diseases, Sahlgrenska University Hospital/Östra, Göteborg, Sweden*
[2]*Department of Internal Medicine, Varberg Hospital, Varberg, Sweden*
[3]*Department of Medical Microbiology, Malmö University Hospital, Malmö, Sweden*
[4]*Department of Infectious Diseases, University Hospital, Linköping, Sweden*
[5]*Department of Infectious Diseases, Karolinska Institute, Huddinge Hospital, Huddinge, Sweden*
[6]*Blood Bank, Sahlgrenska University Hospital/Östra, Göteborg, Sweden*

Sera from 62 hepatitis C virus (HCV)-infected Swedish blood donors were tested by a nested polymerase chain reaction using primers targeting the 5′-noncoding region of the GB virus-C/hepatitis G (GBV-C/HGV) genome and an enzyme-linked immunosorbent assay that detects antibodies to the envelope protein E2 of GBV-C/HGV (anti-E2). Fourteen (22%) and 21 (34%) of the 62 blood donors were found to be GBV-C/HGV RNA and anti-E2 positive, respectively. None of the blood donors was positive for both GBV-C/HGV RNA and anti-E2. Thus, 35 of 62 (56%) HCV-infected donors had been exposed to GBV-C/HGV infection. At sequencing of the 14 GBV-C/HGV isolates, 12 were identified as subtype 2a and 2 as subtype 2b. One of 7 (14%) donors with mild liver disease such as steatosis and nonspecific reactive hepatitis had been exposed to GBV-C/HGV vs. 34 of 55 (62%) with chronic hepatitis with or without cirrhosis (P = 0.04). All other differences in histology were small between HCV and dual HCV GBV-C/HGV-infected donors. In conclusion, more than half of HCV-infected Swedish blood donors in this study were positive for either GBV-C/HGV RNA or anti-E2. GBV-C/HGV viremia and seropositivity were mutually exclusive. ***J. Med. Virol. 54:75–79, 1998.***

KEY WORDS: HCV; HGV; GBV-C; anti-E2; blood donors; chronic hepatitis

INTRODUCTION

GB virus type C (GBV-C)/hepatitis G virus (HGV) are blood-borne viruses infecting humans that have been discovered recently by two independent research groups [Simon et al., 1995; Linnen et al., 1996]. When nucleotide and predicted amino acid sequences were compared, both agents were highly homologous. GBV-C and HGV, isolates of the same virus, are now considered to represent a new species in the Flaviviridae family, distantly related to the hepatitis C virus (HCV). In the present study, the terminology GBV-C/HGV is used.

Recently, enzyme-linked immunosorbent assays (ELISA) have been described [Dille et al., 1997; Tacke et al., 1997]. These tests detect antibodies to the envelope protein E2, which can be found in individuals with resolved GBV-C/HGV viremia. Detection of the GBV-C/HGV agent relies on molecular techniques such as the polymerase chain reaction (PCR). GBV-C/HGV viremia has been found in patients with liver disease of different etiology (hepatitis B and C) and in parenterally exposed individuals (transfused with blood, drug addicts, and hemophiliac and dialysis patients) [Dawson et al., 1996; Linnen et al., 1996; Schleicher et al., 1996; Tacke et al., 1997]. GBV-C/HGV viremia has been found in blood donors tested randomly with normal transaminases and in patients with fulminant hepatitis [Yoshiba et al., 1995; Linnen et al., 1996]. However, the association between GBV-C/HGV and fulminant hepatitis remains controversial [Miyakawa and Mayumi, 1997]. Thus, the potential of the GBV-C/HGV to induce liver disease is by no means clear.

The purpose of the present study was to investigate the occurrence of GBV-C/HGV viremia (and GBV-C/HGV subtypes) and GBV-C/HGV antibodies (anti-E2) in a well-defined group of HCV-infected Swedish blood

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*Correspondence to: Steven Shev, M.D., Ph.D., Department of Infectious Diseases, Sahlgrenska University Hospital/Östra, S-41685 Göteborg, Sweden. E-mail: steven.shev@medfak.gu.se

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donors and to correlate these findings to liver histology, parenteral risk factors, and HCV genotypes.

MATERIALS AND METHODS

Sixty-two (44 men, 18 women) individuals tested by second-generation anti-HCV ELISA (Ortho Diagnostic Systems, Raritan, NJ, or Abbott Laboratories, North Chicago, IL) and found positive for HCV-RNA [Widell et al., 1991] had been investigated previously [Shev et al., 1995b]. Swedish blood donors with a median age of 34 years (range = 22–53) were included into the study. HCV genotyping was based on PCR using type-specific primers directed against the HCV core gene [Widell et al., 1994]. Blood donors were interviewed for potential parenteral risk factors, and the most likely source of infection (intravenous drug use, blood transfusion, tattoos, or unknown) was recorded. All infected donors underwent liver biopsies. Serum alanine aminotransferase (ALT; reference <0.7 μkat/l) was determined with standard laboratory procedures. All blood donors were HBsAg and anti-HIV negative as determined by commercial immunoassays.

Detection of GBV-C/HGV RNA

Viral RNA was extracted from serum as described previously [Widell et al., 1991] using water controls between every sample. A GBV-C/HGV RNA-positive serum, in which the isolate A14 had been verified by sequencing, was diluted 10^{-3}, 10^{-4}, and 10^{-5} and included in each test run. PCR testing was done by nested PCR targeting the 5′-noncoding region (5′-NCR) with primers based on sequence data of full-length genomes published by Abbott and Genelabs [Leary et al., 1996; Linnen et al., 1996]. Primers in the first round of PCR were 5′-CGGCCAAAAGGTGGTGGATG (sense) and 5′-CACTGGTCCTTGTCAACTCG (antisense) and in the second round of PCR 5′-GGTGATGACAGGGTTGGTAG (sense) and GCCTATTGGTCAAGAGA(C-)GACAT (antisense). Products of expected size (250 base pairs) were visualized by ultraviolet light on agarose gels after ethidium bromide staining. A full description of the assay will be published elsewhere [Björkman P, Sundström G, Widell A, et al., In Press].

Sequencing of Amplified GBV-C/HGV Products

GBV-C/HGV PCR fragments were cut out from gels, DNA purified, and subjected to cycle sequencing using inner primers and the Perkin Elmer ABI PRISM TM Dye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq DNA Polymerase, FS. Products were then analyzed on an ABI 373 sequenator, edited, and aligned using the Factura and Sequence Navigator programs applied on both strands. The size of the GBV-C/HGV fragment that was sequenced between the primers was 209 base pairs. Sequences were thereafter compared with the recently published 5′NCR data from Abbott, where subtype-specific mutations for subtypes 1a, 1b, 2a, 2b, and 3 are presented [Muerhoff et al., 1996].

Antibody Test to E2 Protein of GBV-C/HGV

An ELISA introduced recently (Anti-HGenv; Boehringer Mannheim; Penzberg, Germany) was used and interpreted as recommended by the manufacturer.

Histology

Liver biopsies were carried out by the Menghini technique. Routine histological examination was undertaken by pathologists in accordance with international criteria [Bianchi et al., 1977]. The results were classified as normal, steatosis, chronic persistent hepatitis (CPH), chronic active hepatitis (CAH) without or with signs of cirrhosis, and nonspecific reactive hepatitis where only minimal infiltration of inflammatory cells was observed and not fulfilling the criteria for CPH.

Statistical Analysis

Statistical analysis was carried out by using Fisher's exact test and the Mann-Whitney test with two-sided P values. In all tests, $P < 0.05$ was considered to be statistically significant.

The study was approved by the local ethics committees, and all blood donors gave informed consent.

RESULTS

Fourteen of 62 (22%) HCV-infected blood donors, including 6 of the 18 (33%) women and 8 of the 44 (18%) men, were positive for GBV-C/HGV RNA. Twenty-one (16 men, 5 women) of the 62 (34%) donors were positive for anti-E2. GBV-C/HGV viremia and anti-E2 positivity were mutually exclusive. There were no significant differences in age or sex between GBV-C/HGV viremic or seropositive donors and those donors never exposed to GBV-C/HGV.

GBV-C/HGV Subtypes

Sequencing of amplified GBV-C/HGV products showed that 12 of the isolates displayed sequences typical for the 2a subtype and two for the 2b subtype. Hence, none was subtype 1a, 1b, or 3. A small number of divergencies were seen throughout the sequence, with a cluster of changes at position 250–253 using the position numbering according to Muerhoff et al. [1996]. Of the analyzed 14 sequences, only two of the 2a isolates were identical in the analyzed region. The two 2b isolates were also identical in the analyzed region. The remaining 10 isolates showed minor differences in the amplified fragment.

GBV-C/HGV Antibodies (Anti-E2)

In 21 sera that originally tested positive or borderline positive, reactivity remained positive and could be confirmed further when tested in parallel wells with antigen and wells without antigen, thereby giving ratios between 3.3 and 30. In one case, the increased absorbance was the same with and without presence of antigen, indicating nonspecific binding. In four additional sera with borderline values below the cutoff, ratios between 2.4 and 3.8 were seen. These four sera

TABLE I. GBV-C/HGV RNA and Anti-E2 Findings as Correlated to Liver Morphology in 62 Hepatitis C Viremic Blood Donors

Liver morphology	No.	GBV-C/HGV RNA positive (%)	Anti-E2 positive (%)	Total exposed to GBV-C/HGV (%)
Steatosis	4	0	1 (25)	1* (25)
Nonspecific reactive hepatitis	3	0	0	0*
CPH[a]	43	9 (21)	17 (40)	26 (60)
CAH[b]	9	5 (56)	2 (22)	7 (78)
CAH with cirrhosis	3	0	1 (33)	1 (33)
Total	62	14 (22)	21 (34)	35 (56)

[a]CPH, chronic persistent hepatitis.
[b]CAH, chronic active hepatitis.
*P = 0.04 (1/7 vs. 34/55).

were considered to be negative. The rest of the samples, including all PCR-positive samples, were negative.

Exposure to GBV-C/HGV (Ongoing Viremia or Past Infection) as Correlated to Liver Histology

One of 7 (14%) donors with mild liver disease (steatosis or nonspecific reactive hepatitis) as compared with 34 of 55 (62%) with chronic hepatitis (CPH, CAH, or CAH with cirrhosis) had ongoing or resolved GBV-C/HGV infection (P = 0.04) (Table I). All other differences in histology were not significant, including histological differences between donors with HCV only and those with dual HCV–GBV-C/HGV viremia. All three donors with the most severe liver disease (CAH with cirrhosis) were negative for GBV-C/HGV RNA and only 1 was positive for anti-E2.

GBV-C/HGV RNA as Correlated to ALT Levels

There was no significant difference in ALT values (at the time of liver biopsy) between GBV-C/HGV RNA-positive (mean ALT = 1.06 μkat/l) and negative (mean ALT = 1.35 μkat/l) donors or between anti-E2-positive (mean ALT = 1.44 μkat/l) and GBV-C/HGV RNA-positive donors. GBV-C/HGV exposed vs. nonexposed donors had mean ALTs of 1.3 μkat/l and 1.25 μkat/l, respectively. Twelve of 38 (32%) GBV-C/HGV RNA negative as compared with 4 of 14 (29%) GBV-C/HGV RNA positive donors had normal ALT values at the time of biopsy.

GBV-C/HGV RNA as Correlated to HCV Genotype

Thirty-eight percent of blood donors with HCV genotype 2b, 28% with genotype 1a, 17% with genotype 1b, but only 6% with genotype 3a were positive for GBV-C/HGV RNA (Table II).

GBV-C/HGV RNA as Correlated to Parenteral Risk Factors

No significant differences in GBV-C/HGV RNA or anti-E2 positivity were found when comparing donors with different parenteral risk factors (Table III). Seventy-one percent of all HCV-infected donors without known parenteral risk factors had been exposed to GBV-C/HGV.

TABLE II. GBV-C/HGV RNA and Anti-E2 Findings as Correlated to HCV Genotypes Among 62 Hepatitis C Viremic Blood Donors

HCV genotype	No.	GBV-C/HGV RNA positive (%)	Anti-E2 positive (%)	Total exposed to GBV-C/HGV (%)
1a	25	7 (28)	11 (44)	18 (72)
1b	6	1 (17)	1 (17)	2 (33)
2b	13	5 (38)	3 (23)	8 (61)
3a	18	1 (6)	6 (33)	7 (39)

DISCUSSION

In the present study, dual GBV-C/HGV and HCV viremia was found in approximately one-fourth of deferred HCV-infected Swedish blood donors. In other studies, GBV-C/HGV RNA had been detected in 15–25% of patients with acute or chronic hepatitis C [Dawson et al., 1996; Linnen et al., 1996; Schleicher et al., 1996; Alter et al., 1997]. In contrast to the findings of Schleicher et al. [1996], we did not find any age differences between GBV-C/HGV-positive and -negative patients with chronic hepatitis C. The study was conducted among HCV-infected blood donors not identified through illness but through anti-HCV screening and thus with a lower age as compared with patients with chronic hepatitis C as determined by Schleicher et al. [1996].

In the present study, GBV-C/HGV seropositivity and GBV-C/HGV viremia were found to be mutually exclusive. Although rare occurrences of combined GBV-C/HGV and anti-E2 positivity have been found [Dille et al., 1997; Tacke et al., 1997], this is probably due to seroconversion and the clearing of viremia [Dille et al., 1997]. It is clear that at least the present (first generation) antibody tests are of no or marginal value for identifying GBV-C/HGV viremic blood donors.

A significantly greater number of blood donors with chronic hepatitis by liver biopsy had been exposed to GBV-C/HGV (either anti-E2 or GBV-C/HGV RNA positive) as compared with those with steatosis and nonspecific reactive hepatitis. However, comparing donors

TABLE III. GBV-C/HGV RNA and Anti-E2 Results in 62 Hepatitis C Viremic Blood Donors as Correlated to Parenteral Risk Factors

Epidemiology[a]	No.	GBV-C/HGV RNA positive (%)	Anti-E2 positive (%)	Total exposed to GBV-C/HGV (%)
Blood transfusion	12	1 (8)	3 (25)	4 (33)
IVDU[b]	19	3 (16)	7 (37)	10 (53)
Tattoo	7	2 (29)	2 (29)	4 (57)
No known parenteral risk factor	24	8 (33)	9 (38)	17 (71)

[a]Each subject was classified regarding his/her major parenteral risk group, if identified.
[b]IVDU, intravenous drug use.

with steatosis, nonspecific reactive hepatitis, and CPH with those with CAH with or without cirrhosis showed no significant differences. Differences in liver histology between HCV-infected donors with ongoing GBV-C/HGV viremia and those with only HCV viremia were not significant. The histological impact of superinfection with GBV-C/HGV on HCV infection seems to be mild if any.

No significant differences were found in ALT levels between HCV only and HCV–GBV-C/HGV-infected donors. Linnen et al. [1996] detected a similar prevalence of GBV-C/HGV RNA in blood donors with normal and elevated ALT levels, findings that raise questions about the role of GBV-C/HGV in causing chronic liver disease. However, it is known that, in HCV-infected patients, normal ALT levels do not exclude chronic hepatitis by liver biopsy [Shev et al., 1993].

Recent studies have found evidence for, although less than for HCV, GBV-C/HGV genomic heterogeneity [Kao et al., 1996; Muerhoff et al., 1996]. In the present study, the majority of HCV/GBV-C-infected Swedish blood donors had GBV-C/HGV subtype 2a and a minority had subtype 2b, which is in accordance with the findings of Muerhoff et al. [1996] who identified subtypes 2a or 2b in European patients with GBV-C/HGV viremia. The only 2 donors with GBV-C/HGV subtype 2b had CPH on liver biopsy and HCV genotype 1a. It remains to be shown whether different subtypes of GBV-C/HGV differ in pathogenicity.

In the present investigation, donors with HCV genotype 3a were seldom infected chronically with GBV-C/HGV. However, there were no significant differences in presence of anti-E2 in patients with different HCV genotypes. Likewise, Schleicher et al. [1996] could not find any association between dual HCV and GBV-C/HGV viremia and any specific HCV genotype.

The findings of dual HCV–GBV-C/HGV infections among intravenous drug users (IVDU; 16%) were similar to those of an American study among HCV seropositive IVDU [Dawson et al., 1996], whereas German studies [Schleicher et al., 1996; Schreier et al., 1996] detected dual infection in almost half of the IVDU tested. This difference may be due to the fact that the German studies addressed IVDU with chronic hepatitis C, whereas we studied HCV-infected blood donors, some of whom had a more distant history of IVDU. Only 1 of 12 previously transfused with blood was found to be coinfected with GBV-C/HGV. This donor received a blood transfusion 18 years before being enrolled in the present study. The other 11 donors (3 anti-E2 positive) had blood transfusions at a mean of 13 years before being enrolled in the present study.

Sexual transmission of HCV is considered to be uncommon [Shev et al., 1995a]. In a recent study (presented in abstract form), GBV-C/HGV but not HCV was found in seminal fluid in dually infected (HCV–GBV-C/HGV) individuals [Persico et al., 1996]; sexual transmission of GBV-C/HGV may be common. Although not significant, in the present study, more blood donors without known parenteral risk factors had been exposed to GBV-C/HGV infections than were blood donors who had a history of prior blood transfusion or IVDU. Considering the high level of exposure to GBV-C/HGV among donors without any recognized parenteral risk factor for HCV infection, other routes of infection may exist that have not yet been studied. Vertical transmission of GBV-C/HGV has been demonstrated, although dual transmission of HCV and GBV-C/HGV was not found [Feucht et al., 1996]. It is unknown if vertically transmitted GBV-C/HGV infection persists into adult life.

In summary, more than half of the HCV-infected blood donors in the present Swedish study had been exposed to GBV-C/HGV. GBV-C/HGV viremia and anti-E2 positivity was found to be mutually exclusive. Donors with chronic hepatitis seemed to have been exposed more often to GBV-C/HGV virus than those with less advanced liver damage. The route of GBV-C/HGV transmission in blood donors with dual HCV–GBV-C/HGV infections remains only partly known.

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